BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

(12) UK Patent Application (19) GB (11) 2 184 013 (13) A

(43) Application published 17 Jun 1987

(21) Application No 8530637

(22) Date of filing 12 Dec 1985

(51) INT CL⁴
A61K 9/50 31/325

(52) Domestic classification (Edition I)
A5B 180 483 48Y 513 51Y 552 55Y 575 57Y 586 58Y 616 61Y 644 64Y 802 822 829 835 L
U1S 1312 A5B

(56) Documents cited
None

(58) Field of search
A5B
Selected US specifications from IPC sub-class A61K

(71) Applicant
Centralna Laboratoria Po Helmintologia

(incorporated in Bulgaria),

1113 Sofia, Akademik Bonchev Street, Block 25, Bulgaria

(72) Inventors
Evgeni Evgeniev Gabev,
Detchko Kirilov Svilenov,
Olga Todorova Polyakova-Kresteva,
Ivan Vassilev Ivanov,
Tufik Hussein Hassan

(74) Agent and/or Address for Service
Haseltine Lake & Co., Hazlitt House, 28 Southampton Buildings, Chancery Lane, London WC2A 1AT

(54) Anthelminthic composition and method for its preparation

(57) A pharmacologically acceptable anthelminthic composition based on methyl-5-benzoyl-2-benzamidazole carbamate is suitable for use in the treatment of hydatidosis and alveococcosis with high effectiveness, substantial absence of toxicity and suitable for intravenous administration. It is an aqueous liposome suspension whose lipid component is constituted by egg yolk total lipid, whose aqueous phase is constituted by 0.8 to 1% by weight aqueous NaCl and which contains a ratio of active ingredient:egg yolk total lipid:NaCl solution of 0.5 to 2.0:0.2 to 0.6:20 to 80. The composition is prepared by introducing the active ingredient into an organic solvent solution of the egg yolk total lipid, evaporating the solution obtained to dryness, adding the sodium chloride solution and then carrying out a cycle of freezing and melting the composition thereby obtained from 1 to 12 times.

GB 2 184 013 A

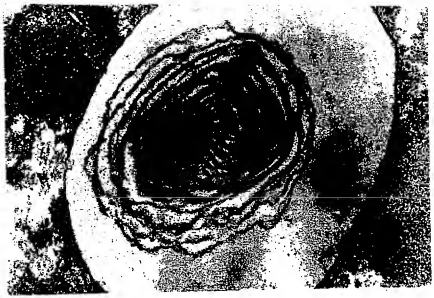

FIG. 1.

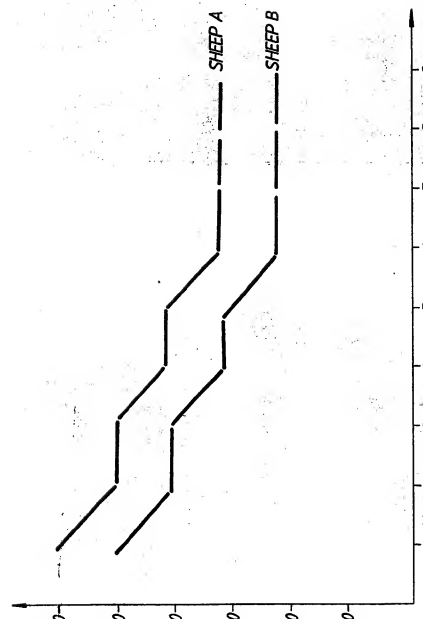

FIG.2.

FIG. 3.

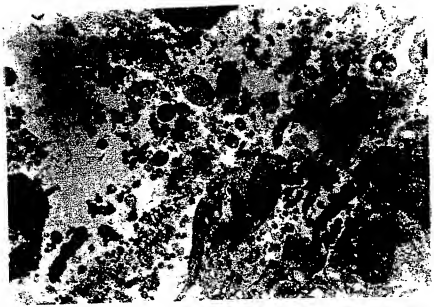

FIG. 4.

SPECIFICATION

Anthelminthic Composition and Method for its Preparation

This invention relates to an anthelminthic composition for use in human and veterinary medicine, in particular for treating hydatidosis (echinococcosis) and alveococcosis, and to a method for the preparation thereof.

It is already known to use mebendazole (methyl - 5 - benzoyl - 2 - benzimidazole carbamate) as a drug in tablet form for treating hydatidosis and alveococcosis (E. Zaharieva et al. "Drugs Reference Book", Sofia 1982, p. 826).

The disadvantages of using mebendazole in such form as an anthelminthic are as follows: low effectiveness with both humans and animals, even when administered each day over a period of from one to five years; toxicity with respect to the receptor organisms; its administration gives rise to disturbances during the final months of pregnancy, this being believed to be due significantly to the form of preparation utilised.

Liposome formulations are known for use in the administration of drugs. These use phospholipids as vectors for the delivery of the biologically active substances to the location at which they are to act. Methods for the preparation of liposomes are described for example by Francis Szoka, Jr. and Demetrois Papahadjopoulos in Ann. Rev. Biophys. Bioeng. 1980, 9, p. 467—508 and in "Liposomes in biological systems", Translation, G. Gregoriadis, A. Alison, Moscow, "Medizine", 1983, p. 384. These methods essentially comprise dissolving phospholipids in an organic solvent containing the hydrophobic active agent in solution. Phospholipids are added in a predetermined concentration after which the solvent is evaporated and then water or a buffer as well as possibly detergents, ethanol, ether etc. are added. The mixture which has then been produced is then subjected to ultra-sonication.

Liposomes prepared according to these procedures tend to have chiefly unilemellar structures rendering them of poor efficiency for including optimal quantities of hydrophobic pharmaceutical substances such as mebendazole. They are therefore not generally suitable for intravenous administration because of the quantities required for them to have the required high effectiveness with respect to parasites.

According to the present invention, there is provided an anthelminthic composition which is in the form of an aqueous liposome suspension whose lipid component is constituted by egg yolk total lipid and which has a trapped aqueous phase which is constituted by an 0.8—1% by weight aqueous solution of sodium chloride, methyl - 5 - benzoyl - 2 - benzimidazole carbamate being attached to hydrophobic centers in the lamellar structures of the liposomes, the liposomes being produced from their components utilising in the weight ratio of methyl - 5 - benzoyl - 2 - benzimadazole carbamate:egg yolk total lipid:aqueous solution of NaCl of 0.5 to 2.0:0.2 to 0.6:20 to 80.

This invention also provides a method for the production of an anthelminthic composition which comprises dissolving egg yolk total lipid in an organic solvent, preferably a chloroform-methanol solution, introducing the methyl - 5 - benzoyl - 2 - benzimidazole carbamate into the solution obtained, evaporating the solution obtained to dryness at a temperature of preferably from 20 to 40°C, adding an 0.8—1% by weight aqueous solution of sodium chloride to the dry residue with stirring and then carrying out a cycle of freezing and melting the composition thereby obtained from 1 to 12 times, preferably utilising temperatures of −196°C for freezing and up to +35°C for melting, the weight ratios of the methyl - 5 - benzoyl - 2 - benzimidazole carbamate:egg yolk total lipid:aqueous NaCl solution utilised being 0.5 to 2.0:0.2 to 0.6:20 to 80.

The liposomes thus prepared are homogeneous insofar as average diameter is concerned, this having been determined by electron microscopy to be, on average, 0.4 microns, with maximum and minimum of 0.3 and 0.5 microns. The liposomes have a multi-lamellar structure, being built up of several bimolecular layers.

When producing the anthelminthic compositions by the method of this invention, the aqueous solution of sodium chloride which is preferably an 0.9% by weight solution (physiological saline) is generally added at ambient temperature and with constant stirring in order to hydrate the lipid. The weight ratio of aqueous phase:mebendazole:lipid is preferably in the range of from 15:0.6:0.1 to 25:0.5:0.2. As a result of this, the liposome form anthelminthic composition is obtained in a form particularly suitable for intravenous administration.

The advantages of the anthelminthic composition according to the invention insofar as the treatment of for example hydratidosis and alveococcosis is concerned are the high effectiveness of the composition, the short period of treatment needed and its minimal toxicity. The prolonged activity achieved with a liposome or form of composition enables there to be a reduction in the frequency of administration and in the total dosage. The anthelminthic composition is biodegradable.

The aforesaid method according to the invention for the production of liposomic anthelminthic compositions involves the absence of need for special apparatus and special starting materials. It can be rapidly and simply carried out to yield stable liposomes.

The following Example illustrates the invention:—

EXAMPLE

Preparation of the Composition

An anthelminthic composition according to the invention was prepared as follows. 3.2 ml of a solution of egg yolk total lipid dissolved in distilled chloroform containing 1% by volume of methanol were added under sterile conditions to a round bottomed flask by means of a pipette. The initial lipid solution had a lipid concentration of 129 mg/ml. 10 ml of chloroform were then added and the contents of the flask were shaken vigorously. 1.0 g of mebendazole was added gredually to the lipid

solution whil continuing shaking. The s lvent was then evaporated off over 5 to 6 minutes in a rotor type evaporator at ambient temperature. A further 5 ml of chloroform were then added to the residue and the contents of the flask were subjected to evaporation in the rotor evaporator over 1.5 hours at a temperature of 35±2°C (water bath). The last possible traces of chloroform were eliminated by blowing out with nitrogen for 5 minutes.

40 ml of sterile physiological saline (0.9% aqueous solution of sodium chloride) were added to the dry residue. Nitrogen was then bubbled through the suspension obtained for 5 minutes after which the suspension was subjected to incubation for one hour at ambient temperature while undergoing constant stirring. The suspension was then frozen within 2 to 3 minutes using liquid nitrogen and subsequently was melted in e water bath adjusted to 50°C within 2 to 3 minutes while continuously carrying out intensive shaking. The temperature of the contents of the flask was controlled so as not to exceed 40°C.

The anthelminthic composition thereby produced had the following composition: aqueous phase (0.9% aqueous solution of sodium chloride):mebendazole:lipid phase of 40 ml:1 g:0.4 g.

Appearance of Composition.

Electron microscopic examination of the product of the aforesaid preparative procedure indicated the precise characteristics of the liposomes produced. The liposomes obtained were, as can be seen from the accompanying Figure 1, vesicular, multi-lamellar bilayer lipid membrane structures. It can be seen that a liposome is built up from a plurality of lamellae, each lamella representing a bimolecular lipid layer with a thickness of about 500 nm, i.e. corresponding to the thickness of natural biological membranes.

Activity

The anthelminthic composition produced as aforesaid was tested on animals experimentally infected with hydatidosis. Two groups of sheep, groups A and B, were selected for the experimentation which commenced 20 months after infection. In order to follow the course of treatment, e blood sample was taken prior to each administration of the anthelminthic composition with the object of studying the dynamics of antibody formation according to the RPH (reaction of passive hemagglutination) method. The recorded dynamics are shown in the accompanying Figure 2. During the course of treatment, a check was made on the general status of the animals and in particular in respect of macroscopic, image and pathohistologic changes in the parasite and in the internal rgans of the host as well as on ultrastructural changes in the parasite and in the affect d rgans.

Administrations followed the following procedur . Immediately after pr paration f th liposomes incorporating mebendazole which wer in the form of a milky white aque us susp nsion having a specific odour and a salty flavour, the suspension of liposom s was injected in an amount of 20 ml per dose and providing an amount of mebendazole of 10 mg/kg, administration taking place once every week by intravenous injection into the jugular vein. The rate at which the suspension was administered did not appear to effect tolerance toward the preparation, whether administration took place within 5 to 6 seconds or 4 to 5 minutes. No harmful side effects were observed during the entire period of treatment. The course of treatment was constituted by six injections.

Three weeks after the last administration of the liposomes containing mebendazole, the animals which had been treated were sacrificed, the animals having been given one further injection one hour prior to sacrifice with a view to enabling electron microscopic follow up of liposomes utilisation in the organs to be carried out. A control group of animals infected with the same parasite but which had not been submitted to the mebendazole-liposome treatment was also subjected to histological and electron microscopic investigation.

At the completion of the investigation of all the animels, it was seen that the treated animals had a very good general status and increased weight. As can be seen from Figure 2, the antibody titre had begun to decrease progressively after the second administration up until the sixth treatment and then was stabilized and remained constant up to the end of the period studied, that is nine weeks after the beginning of experimentation. Indeed, parallel behaviour was noted with the two groups of sheep which had been infested. In the case of the control animals, however, it was seen in macroscopic examination that parasitic cysts dimensionsed 10 to 20 mm with a cherecteristic macroscopic pettern and ultrastructure were present. In contrast, the cysts in the treated animals had dimensions of 1 to 5 mm and pathohistological examination enabled it to be seen that there had been a complete destruction of the germinative layer and considerable changes in the cuticular membrane. The character of the breakdown of the cysts is well shown in the accompanying Figure 3. Calcium precipitations are observable pericystically in the wall and in several cases also in the echinococcosis vesicles themselves. The internal organs of the treated animals otherwise do not indicate any deviations from normal. Ultrastructural examination showed that in the treated animels there wes a well conserved parenchyma. Indeed a multitude of secondary liposomes with the characteristics of autophagosomes and residual corpuscles were observed. At some locations, residues of liposomes submitted to destruction and phagocytosis could be identified. The germinative membrane showed a complete destruction leaving residues in th form of vesicles, membranic formations, osmiphylic granules etc. as shown in the accompanying Figur 4. Inclusions with characteristics f liposomes submitted to d comp sitions could be s en in the necrosis zones.

CLAIMS

1. An anthelminthic composition which is in th form of an anthelminthic composition which is in th form f an aqueous liposome susp nsi n wh se lipid component is constituted by egg yolk total lipid and which has a trapped aqueous phase which is constituted by an 0.8—1% by weight aqueous solution of sodium chloride, methyl - 5 - benzoyl - 2 - benzimidazole carbamate being attached to hydrophobic centres on the lamellar structures of the liposomes, the liposomes being produced from their components utilised in the weight ratio of methyl - 5 - benzoyl - 2 - benzimadazole carbamate:egg yolk total lipid:aqueous solution of NaCl of 0.5 to 2.0:0.2 to 0.6:20 to 80.

2. An anthelminthic composition according to claim 1, wherein the aqueous solution of NaCl is an 0.9% by weight solution of NaCl.

3. An anthelminthic composition as claimed in claim 1 or 2, wherein the weight ratio of aqueous phase:mebendazole:lipid is from 15:0.6:0.1 to 25:0.5:0.2.

4. An anthelminthic composition, substantially as described in the foregoing Example.

5. A method for the production of an anthelminthic composition which comprises dissolving egg yolk total lipid in an organic solvent, introducing the methyl - 5 - benzoyl - 2 - benzimidazole carbamate into the solution obtained, evaporating the solution obtained to dryness, adding an 0.8—1% by weight aqueous solution of sodium chloride to the dry residue with stirring and then carrying ut a cycle of freezing and melting the composition thereby obtained from 1 to 12 times, the weight ratios of the methyl - 5 - benz yl - 2 - b nzimidazole carbamate:egg y lk total lipid:aqueous NaCl solution utilised being 0.5 to 2.0:0.2 to 0.6:20 to 80.

6. A method as claimed in claim 5, wherein the organic solvent is a mixture of chloroform and methanol.

7. A method as claimed in claim 6, wherein the evaporating to dryness is carried out at from 20 to 40°C.

8. A method as claimed in claim 5, 6 or 7, wherein the aqueous solution of NaCl is an 0.9% by weight solution of NaCl.

9. A method as claimed in any one of claims 5 to 8, wherein the aqueous solution of NaCl is sterile physiological saline.

10. A method as claimed in any one of claims 5 to 9, wherein the aqueous solution of sodium chloride is added at ambient temperature.

11. A method as claimed in any one of claims 5 to 10, wherein temperatures of −196°C and up to +35°C are utilised for the freezing and melting respectively.

12. A method for the production of an anthelminthic composition, substantially as described in the foregoing Example.

13. An anthelminthic composition, whenever produced by the process claimed in any one of claims 5 to 12.

Printed for Her Majesty's Stationery Office by Courier Press, Leamington Spa, 6/1987. Demand No. 8991685.
Published by the Patent Office, 25 Southampton Buildings, London, WC2A 1AY, from which copies may be obtained.